Remarks

The Amendments

Claims 1 and 23 have been amended to recite that "the first primer terminates at its 5' end with a 5' portion" in place of comprises "a 5' portion." The amendment is supported by the specification which discloses that the probe on the solid support "is identical in complementarity to a tag **at the 5' end** of the ASPCR primer." (Page 3, lines 12-14, emphasis added; See also Figure 1.) Thus the amendment introduces no new matter.

Claims 4-7 have been amended to properly be dependent on claim 2 in place of claim 1.

Claims 26-29 have been amended to properly be dependent on claim 24 in place of claim 23.

The Rejection of Claims 1, 2, 4, 6, 8-11, 13, 23, 24, 26, 28, 30-33, and 35 Under 35 U.S.C. § 102(b)

Claims 1, 2, 4, 6, 8-11, 13, 23, 24, 26, 28, 30-33, and 35 are rejected as anticipated by Whitcombe *et al.* (WO 97/42345; "Whitcombe"). The rejection is respectfully traversed.

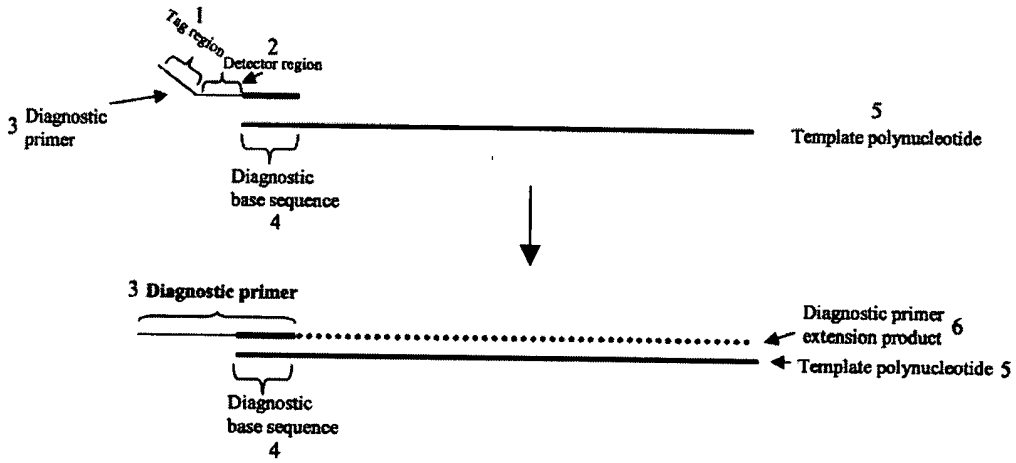
Claims 1 and 23 are the independent claims of the rejected claim set. Claim 1 is directed to a method to determine a nucleotide at a polymorphic locus in a nucleic acid sample. The method comprises four steps: (i) amplifying a region of DNA comprising a polymorphic locus in the sample; (ii) labeling the amplified DNA products; (iii) hybridizing the labeled amplified DNA products to a probe on a solid support; and (iv) detecting the hybridized labeled amplified DNA products. The amplification is carried out using a pair of primers. The first primer terminates at its 3' end at the polymorphic locus and terminates at its 5' end with a 5' portion that

is identical in sequence to all or part of a probe on a solid support and not complementary to the region of DNA. The step of amplifying produces a first strand and a second strand. The first strand comprises a portion identical to all or part of the probe, and the second strand comprises a 5' portion complementary to all or part of the probe.

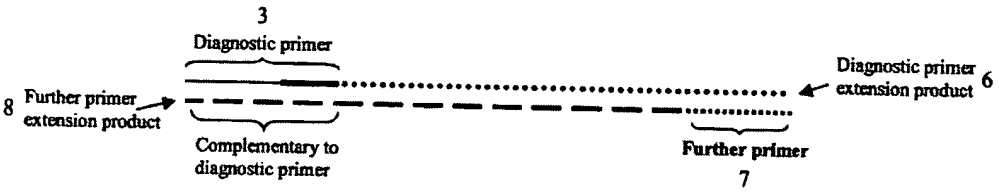
Claim 23 is directed to a method to prepare samples for analysis to determine a nucleotide at a polymorphic locus in a nucleic acid sample. The method recites the steps of amplifying, labeling, and hybridizing as in claim 1, but does not recite the step of detecting.

Whitcombe does not expressly or inherently teach each and every element as set forth in the methods of claims 1, 2, 4, 6, 8-11, 13, 23, 24, 26, 28, 30-33, and 35.

Whitcombe teaches methods for detecting a target nucleic acid sequence in a sample of polynucleotides. The methods employ primers and polymerization of nucleotides. A tailed diagnostic primer (3) is hybridized to a diagnostic base sequence (4) and extended on a template polynucleotide (5) to produce a diagnostic primer extension product (6). The tail of the diagnostic primer comprises a tag region (1) and a detector region (2). (Page 2, lines 1-6.)

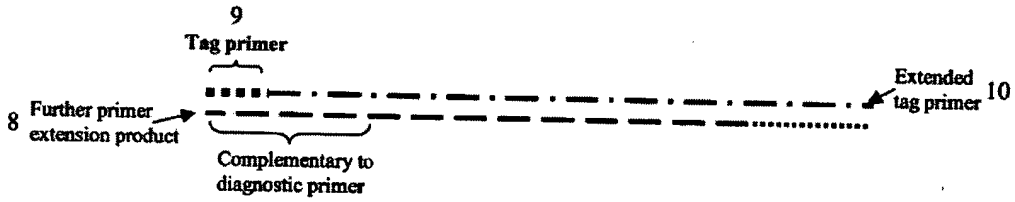


A further primer (7) is hybridized to the diagnostic primer extension product (6) and extended to produce a further primer extension product (8). (Page 2, lines 6-7.)



The sequence complementary to the detector region of the diagnostic primer in the further extension product (8) can be detected. "The detector region in the further extension product may be detected." (Page 2, lines 11-12.) One of many possible modes of detection is via a probe on a solid support. (Page 3, lines 1-2.)

Additional steps can optionally be performed. A tag primer (9) can be hybridized to the further primer extension product (8) at the sequence complementary to the tag region of the diagnostic primer and extended (Page 2, lines 7-9.)



This product (the extended tag primer; 10) can be amplified using the tag primer (9) and the further primer (7). (Page 4, lines 5-6.)

None of the primers disclosed by Whitcomb (the diagnostic (3), the further (7), or the tag (9) primer) "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe on a solid support" as recited in claims 1 and 23.

The diagnostic primer (3) terminates at its 5' end in a tag region (1). The tag region has a sequence identical to a tag primer (9). "The sequence of the tag primer is conveniently identical

to the sequence of the tag region." (Page 3, lines 20-21.) The tag region of the diagnostic primer, upon subsequent polymerization, generates an annealing site for the tag primer. This occurs when a second DNA strand (further extension product; 8) is synthesized that is complementary to the diagnostic primer extension product. This further extension product (8) contains a portion that is complementary to the tag region (1) to which the tag primer (9) can anneal. This portion, however, is not identical to a probe on a solid support. The detector region (2), *i.e.*, the middle portion of the diagnostic primer (3) can be identical to a probe on a solid support. "The sequence of the detector probe need not be the same but is conveniently identical to the sequence of the detector region in the tail [of the diagnostic primer]." (Page 3, lines 7-8.) The detector region (2) generates, upon subsequent polymerization, a hybridization site for a probe in the further extension product (8). "A diagnostic base sequence is identified by reference to the sequence complementary to the detector region." (Page 1, lines 26-27.) Thus the region of the diagnostic primer identical to a probe is the middle region, not the 5' terminal end. Whitcomb's diagnostic primer does not terminate at its 5' end with a 5' portion which is identical in sequence to all or part of a probe on a solid support.

The further primer (7) only contains a nucleic acid sequence that is complementary to the diagnostic primer extension product (6). A "further primer . . . hybridises to a locus at a distance from the diagnostic base sequence." (Page 2, lines 6-7.) Alternatively, the further primer contains a 5' tag region, a middle detection region, and a 3' region that hybridizes to genomic DNA: "We now disclose use of diagnostic and further primers which are genome specific at their 3'-termini but which carry a detector region and common extensions (tags) at their 5' termini." (Page 4, lines 22-24.) Thus, at most, the further primer contains a 5' terminal portion

that is identical to a tag primer and a middle portion that is identical to a probe on a solid support. Whitcombe's further primer does not terminate at its 5' end with a 5' portion which is identical in sequence to all or part of a probe on a solid support.

The tag primer (9) has a sequence identical to the tag region (1) of the diagnostic primer (3) and/or the further (7) primer. "The sequence of the tag primer is conveniently identical to the sequence of the tag region of the tail [of the other primers]." (Page 3, lines 20-21.) Thus the tag primer has no portion that is identical in sequence to all or part of a probe on a solid support.

Whitcombe does not expressly or inherently teach a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe on a solid support." Thus Whitcombe does not expressly or inherently teach each and every element of claims 1 and 23. Claims 2, 4, 6, 8-11, 13, 24, 26, 28, 30-33, and 35 are dependent on claims 1 and 23 and thus also contain this recitation. Withdrawal of this rejection to claims 1, 2, 4, 6, 8-11, 13, 23, 24, 26, 28, 30-33, and 35 is respectfully requested.

The Rejection of Claims 3 and 25 Under 35 U.S.C. § 103(a)

Claims 3 and 25 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Whitcombe et al. (WO 97/42345) in view of Hames et al. (Nucleic Acid Hybridization: a practical approach, 1998, pp. 35, 36, and 42-44). Applicants respectfully traverse.

The combination of Whitcombe and Hames fails to teach or suggest all elements of claims 3 and 25.

Claims 3 and 25 are dependent on claims 1 and 23, and thus include as an element a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe on a solid support."

As discussed above, Whitcombe does not teach a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe on a solid support." Whitcombe teaches a diagnostic primer that comprises a 5' portion that has a sequence identical to a tag primer, a middle portion that is identical to a detector probe, and a 3' portion that is complementary to the target polynucleotide. (See Fig. 9A and its description at page 11, lines 19-21.)

Whitcombe also does not suggest modifying the diagnostic primer such that its 5' end terminates with a 5' portion that is identical to a probe. In fact, Whitcombe teaches that it is advantageous for the diagnostic primer to terminate at its 5' end with a 5' portion identical to a tag primer. Whitcombe teaches that the 5' terminal tag region of the diagnostic primer reduces artifacts introduced during amplification reactions. "Thus whilst primer dimers and other inter-primer artifacts could occur during first phase diagnostic priming, these cannot be amplified during subsequent rounds of tag specific priming." (Page 4, lines 25-27, emphasis added.) Whitcombe also teaches that the 5' terminal tag region reduces experimental costs because different diagnostic primers, which anneal to different diagnostic base sequences, can be designed to contain the same tag region. Thus all the different diagnostic base sequences can be amplified with a single tag primer in a single subsequent reaction. "The use of a common tag primer and common tail sequences has significant cost advantages for a typical assay." (Page 3, lines 24-25.) Furthermore, Whitcombe teaches that if multiple diagnostic base sequences are

amplified in a single reaction using a single tag primer, the amplification efficiencies of each of the different templates are more equal than if multiple sets of primers are used to amplify the different templates. "We have found that the use of identical tag sequence can be advantageously used to even out the efficiencies of different amplification reactions." (Page 4, lines 11-12.) Thus Whitcombe teaches that having a 5' terminal portion of a diagnostic primer reduces amplification artifacts, costs of amplification reactions, and uneven amplification in reactions of more than one product. Whitcombe provides no suggestion to produce a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe."

Hames does not rectify this deficiency of Whitcombe. Hames merely teaches scientific protocols that are used to perform terminal transferase reactions.

Thus the combination of Whitcombe and Hames does not teach or suggest a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe." The combination of Whitcombe does not teach or suggest all the elements recited in claims 3 and 25 and a *prima facie* case of obviousness has not been made. Withdrawal of this rejection to claims 3 and 25 is respectfully requested.

The Rejection of Claims 5, 14-16, 27, and 36-38 Under 35 U.S.C. § 103(a)

Claims 5, 14-16, 27, and 36-38 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Whitcombe et al. (WO 97/42345) in view of Harris et al. (WO 94/02634). Applicants respectfully traverse.

It is respectfully submitted that the combination of Whitcombe and Harris does not teach or suggest all the claim recitations of claims 5, 14-16, 27, and 36-38.

Claims 5, 14-16, 27, and 36-38 are dependent on claims 1 and 23. Thus claims 5, 14-16, 27, and 36-38 include as an element a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe."

Whitcombe does not teach or suggest a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe." Whitcombe teaches a diagnostic primer that comprises a 5' portion that has a sequence identical to a tag primer and a middle portion that is identical to a detector probe. (See Fig. 9A and its description at page 11, lines 19-21.) Whitcombe also teaches that it is advantageous for the diagnostic primer to terminate in a 5' portion that is identical in sequence to a tag primer, and thus does not suggest a primer that terminates in a 5' portion which is identical in sequence to all or part of a probe. Whitcombe teaches that a diagnostic primer that terminates in a 5' portion identical in sequence to a tag primer reduces amplification artifacts during PCR (page 4, lines 25-27), reduces the cost of amplifying multiple diagnostic base sequences (page 3, lines 24-25), and evens out the efficiencies of amplification of multiple diagnostic base sequences in a single reaction (page 4, lines 11-12).

Harris also fails to teach a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe" and thus does not rectify this deficiency of Whitcombe. Harris teaches methods of detecting amplified target nucleic acids. Target nucleic acids are amplified with primers. During amplification, a detector tag is introduced into the amplification product. The detector tag is a label that permits detection of the amplified product. "The detector tag is, for example, a biotin group (labeled as B in the diagram above), which is introduced via a biotinylated primer oligodeoxynucleotide sequence or analogue capable of

being incorporated into the nucleic acid product during amplification." (Page 6, line 17 to page 7, line 1; See also page 35, line 8 through page 36, line 13.) A probe detects the labeled amplification products.

Harris teaches that probes hybridize to amplified nucleic acids at sites not close to where the amplification primers anneal. "The product target site [probe hybridization site] is away from primer sequences and their complements." (Page 14, lines 8-10.) Thus the primers used in these amplification reactions do not contain sequences that participate in hybridization of the product to the probe. These primers do not terminate at their 5' end with a 5' portion which is identical in sequence to all or part of a probe on a solid support. Alternatively, Harris teaches that the probes themselves hybridize to the amplification primers. "[F]or methods where the amplified product is formed only from the primers (e.g. Ligase Chain Reaction, LCR), then the product target sequence [probe hybridization site] will be in part of one strand of two joined primers and will span across the joining point." (Page 14, lines 10-14.) Thus a part of the sequence of the primer is complementary to the probe on a solid support. These primers do not terminate at their 5' end with a 5' portion that is identical in sequence to all or part of a probe.

Thus the combination of Whitcombe and Harris does not teach or suggest a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe." The combination of Whitcombe and Harris does not teach or suggest all the elements recited in claims 5, 14-16, 27, and 36-38 and a *prima facie* case of obviousness must fail. Withdrawal of this rejection to claims 5, 14-16, 27, and 36-38 is respectfully requested.

The Rejection of Claims 7 and 29 Under 35 U.S.C. § 103(a)

Claims 7 and 29 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Whitcombe et al. (WO 97/42345) in view of Vary et al. (U.S. Patent 4,851,331). Applicants respectfully traverse.

It is respectfully submitted that the combination of Whitcombe and Vary does not teach or suggest all the claim recitations of claims 7 and 29.

Claims 7 and 29 are dependent on claims 1 and 23. Thus claims 7 and 29 include as an element a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe."

Whitcombe, as discussed above, does not teach a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe." Whitcombe teaches a diagnostic primer that comprises a 5' portion that has a sequence identical to a tag primer and a middle portion that is identical to a detector probe. (See Fig. 9A and its description at page 11, lines 19-21.) Whitcombe also does not suggest that the diagnostic primer terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe. Rather, Whitcombe teaches that having a tag region at the terminal 5' portion of the diagnostic primer is critical to reducing amplification artifacts during PCR (page 4, lines 25-27), reduces the costs of amplifying multiple diagnostic base sequences (page 3, lines 24-25), and evens out the efficiencies of amplifying multiple diagnostic base sequences in a single reaction (page 4, lines 11-12).

Vary fails to remedy this defect of Whitcombe. Vary teaches a method of detecting a target nucleotide sequence in the nucleic acids of a biological sample. The Patent Office has

acknowledged that Vary does not teach the primer pair wherein the first primer comprises a 5' portion which is identical in sequence to all of a probe on a solid support. (Paper 19, page 3, lines 7-9.)

Thus the combination of Whitcombe and Vary does not teach or suggest a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe." The combination of Whitcombe and Vary fails to teach or suggest all the elements recited in claims 7 and 29. Withdrawal of this rejection of claims 7 and 29 is respectfully requested as the *prima facie* case of obviousness must fail.

The Rejection of Claims 12 and 34 Under 35 U.S.C. § 103(a)

Claims 12 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Whitcombe et al. (WO 97/42345) in view of Brown et al. (U.S. Patent 5,807,522). Applicants respectfully traverse.

It is respectfully submitted that the combination of Whitcombe and Vary does not teach or suggest all the claim recitations of claims 7 and 29.

Claims 12 and 34 are dependent on claims 1 and 23. Thus claims 7 and 29 include as an element a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe."

Whitcombe does not teach a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe." Whitcombe teaches a diagnostic primer that comprises a 5' portion that has a sequence identical to a tag primer and a middle portion that is identical to a detector probe. (See Fig. 9A and its description at page 11, lines 19-21.)

Whitcombe also does not suggest that the diagnostic primer terminate at its 5' end with a 5' portion which is identical in sequence to all or part of a probe because Whitcombe teaches there are advantages to having a tag region at the terminal 5' portion of the primer. (See page 4, lines 25-27; page 3, lines 24-25; and page 4, lines 11-12.)

Brown also does not teach or suggest a primer that "terminates at its 5' end with a 5' portion which is identical in sequence to all or part of a probe" and thus fails to remedy this defect of Whitcombe. Brown teaches the use of microarrays to examine gene expression in *S. cerevisiae*. (Example 1, column 16, line 3, to column 17, line 40.) *S. cerevisiae* chromosomal DNA is amplified and hybridized to an array of probes. The primers used to amplify the *S. cerevisiae* chromosomal DNA had a random nucleotide sequence; the *S. cerevisiae* DNA was "randomly amplified." (Column 16, lines 45-46.) The amplified *S. cerevisiae* DNA was hybridized to probes on the solid support. The probes "were randomly amplified PCR products using physically mapped lambda clones of *S. cerevisiae* genomic DNA as templates." (Column 16, lines 10-13, citations omitted.) Thus the probes have a sequence that is complementary to the primer and yeast chromosomal DNA. Brown does not teach a primer that "terminates in a 5' portion which is identical in sequence to all or part of a probe on a solid support."

Thus the combination of Whitcombe and Brown does not teach or suggest a primer that "terminates in a 5' portion which is identical in sequence to all or part of a probe on a solid support." The combination of Whitcombe and Brown does not teach or suggest all the elements recited in claims 12 and 34 and the *prima facie* case of obviousness must fail. Withdrawal of this rejection to claims 12 and 34 is respectfully requested.

Respectfully submitted,

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